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A NEW METHOD FOR THE DETERMINATION OF THE FOOD VALUE OF PROTEINS, WITH APPLICATION TO CYNOSCION REGALIS.

By GEORGE F. WHITE and ADRIAN THOMAS.

It is frequently desired to compare the rate and course of digestion of various proteins by enzymes according to methods which, while not complicated, will give distinctive and reliable results. Van Slyke's[a] method for the determination of amino acids has been applied by White and Crozier[b] with evidently great success to a comparison of the tryptic proteolysis of beef and several fish meats. The ease of manipulation of the apparatus, the brief time required for a determination, and the regularity of the experimental data, make the process generally useful. The conclusions drawn also conform with those from metabolism experiments, making the results of still greater value.

The method which Sörensen[c] has proposed for the estimation of amino acids and polypeptides, by titration with caustic soda after the addition of formaldehyde, by its simplicity suggests itself for artificial digestion processes. It is the object of this article to show its application to the hydrolysis of *Cynoscion regalis* (squeteague, weakfish) by trypsin, and to compare the results with those obtained by Van Slyke's method.

The squeteague was boiled in water for a quarter of an hour, allowed to drain from excess of liquid, and preserved ice-cold. An analysis of two samples gave an average of 4.52 per cent nitrogen.

The digestion was carried on in 250 c. c. volumetric flasks placed in a thermostat kept at temperature of 37.5° C. Enough meat to furnish 1.5 g. of nitrogen was weighed out, ground up with water together with 1 g. of trypsin, and this mixture poured into the flask; 25 c. c. of N/10 sodium hydroxide solution was added, and the whole made up to 250 c. c. with water. Trypsin is presumably most active in a medium made alkaline with sodium carbonate, but the presence of this salt would interfere with the titration for the amino acids where phenolphthalein must be used as an indicator, so that the alkalinity was insured by the presence of the hydrate. Separate mixtures were made

[a] Van Slyke, D. D.: A method for quantitative determination of aliphatic amino groups. Journal of Biological Chemistry, vol. IX, p. 185–204, 1911. Baltimore.

[b] White, G. F. & Crozier, W.: Comparative proteolysis experiments with trypsin. Journal of the American Chemical Society, vol. 33, p. 2042–2048, 1911. Easton, Pa.

[c] Sörensen, S. P. L.: Fermentstudien. Biochemische Zeitschrift, bd. 7, p. 45–101, 1907. Berlin.

and analyses run in duplicate for the time periods of 1, ½, 2, 5, and 8 hours, respectively. A sample similar to the above but containing no trypsin was also prepared and analyzed with the others according to the following method: At the end of the desired time of digestion, the mixtures were filtered and aliquot portions of the filtrate taken for the various tests; 10 c. c. were used for the determination of total soluble nitrogen by the Kjeldahl method; 10 c. c. for the amino nitrogen by Van Slyke's method; 20 c. c. were treated with 10 c. c. of 40 per cent formaldehyde solution and titrated to a distinct pink color with N/10 sodium hydroxide solution. We found that in every case a distinct end point was obtained, duplicate analyses agreeing within 0.2 per cent. Finally, 10 c. c. of the filtrate from the digested fish were completely hydrolyzed by prolonged digestion on the water bath with 40 c. c. of concentrated hydrochloric acid. This solution was evaporated to dryness, made up to 50 c. c. with water, 10 c. c. tested for amino nitrogen by Van Slyke's method, and 20 c. c. analyzed for amino acids by Sörensen's method.

All the results as obtained above were corrected for amino nitrogen before and after complete hydrolysis, for total soluble nitrogen, and for amino acids as determined by titration with caustic soda, by carrying through the same experiments with trypsin and alkali, but with no protein. Correction was also made for the alkali required to neutralize the formaldehyde solution.

In table 1 are presented the results obtained by applying Van Slyke's method to the tryptic proteolysis of squeteague. The average size of the peptides was calculated by dividing the amount of amino nitrogen present after complete hydrolysis with hydrochloric acid by that in the solution before such hydrolysis. The last two columns of data show the increase with time of proportion of soluble to insoluble nitrogen and of amino to total soluble nitrogen, respectively. The average results of duplicate analyses are given.

TABLE I.—TOTAL AND AMINO NITROGEN IN SOLUTIONS OF CYNOSCION REGALIS HYDROLYZED BY TRYPSIN.

Time in hours.	Soluble nitrogen.	Insoluble nitrogen.	Amino nitrogen.	Amino nitrogen after hydrolysis.	Average size of peptids.	Soluble $\frac{100\times}{\text{total nitrogen.}}$	Amino $\frac{100\times}{\text{soluble nitrogen.}}$
0	0.170	1.330	0.017	0.056	3.29	11.32	10.00
½	1.115	.385	.230	.464	2.02	74.32	20.63
1	1.175	.340	.250	.464	1.86	78.34	21.27
2	1.173	.327	.289	.464	1.61	78.20	24.63
5	1.361	.139	.357	.....	.....	90.72	26.23
8	1.432	.068	.406	.646	1.59	95.45	28.35

In table II are given the average results of the analysis of the proteolyzed solutions according to Sörensen's method. In column 2 the figures represent cubic centimeters of N/10 sodium hydroxide solution required for neutralization after addition of formaldehyde solution. Column 3 is the same for the solutions after complete hydrolysis with hydrochloric acid. The next column gives the ratio of the latter to the former. In

column 5 are given figures for amino nitrogen calculated from the data in column 2, while the values in the last column were obtained by calculating the per cent calculated amino nitrogen of the total soluble nitrogen.

TABLE II.—ANALYSIS BY SÖRENSEN'S METHOD OF SOLUTIONS OF CYNOSCION REGALIS HYDROLYZED BY TRYPSIN.

Time in hours.	Quantity N/10 NaOH required.	Quantity N/10 NaOH required after hydrolysis.	Average size of peptids.	Amino nitrogen calculated.	Amino nitrogen $100 \times \frac{\text{amino nitrogen}}{\text{soluble nitrogen}}$.
	c. c.	*c. c.*			
0	24.85	125.0	5.02	0.035	20.52
¼	182.8	536.8	2.94	.257	23.02
1	213.8	598.0	2.80	.300	25.87
2	232.9	.....	.....	.327	27.87
5	293.5	776.0	2.64	.412	30.28
8	314.8	787.1	2.50	.442	30.87

From table 1 it is seen from the ratio of the soluble to the total nitrogen that the fish meat goes very rapidly into solution, 74.32 per cent of the nitrogen being in solution at the end of a half hour's digestion. Solution, however, is not complete in eight hours' time, a fact which is apparently not in harmony with the results of White and Crozier,[a] who found with the proteins they studied that all the nitrogen was in the soluble form in four to eight hours. The trypsin used, a commercial sample, was of the same activity in both series of experiments. These latter experiments were carried on in a medium made alkaline with sodium carbonate, while the experiments described in this article required sodium hydroxide for reasons already stated. Schierbeck[b] has proved that the action of trypsin in digesting proteins is accelerated by the presence of carbon dioxide in solutions which are slightly alkaline, and it is very probably at least partly due to this fact that the above differences are found. The variation of the proportion of soluble to total nitrogen with increase of time is shown graphically in figure 1. Extrapolation of the curve would indicate that the squeteague would be completely dissolved in about 14 hours.

White and Crozier have shown that their artificial digestion experiments gave results agreeing closely with those obtained by metabolism work with dogs, rates of digestion of different proteins being in the same ratio to each other. Van Slyke and White,[c] in a study of the relation between the digestion and the retention of ingested proteins, found that squeteague is digested more slowly than either beef or cod. From the above facts it is fair to conclude that the tardy solution of the squeteague by trypsin shown by our data is not alone due to the absence of carbon dioxide, but is a consequence of the inherent nature of the protein itself.

[a] White & Crozier, op. cit.

[b] Schierbeck, N. P.: Ueber den einfluss der kohlensaure auf die diastatischen und peptonbildenden fermente im thierischen organismus. Skandinavisches Archiv fur Physiologie, bd. 3, s. 344–375, 1892. Leipzig.

[c] Van Slyke, D. D. & White, G. F.: The relation between the digestibility and the retention of ingested proteins. Journal of Biological Chemistry, vol. IX, p. 219–229, 1911. Baltimore.

The amino nitrogen in solution increases of course with length of time. The average size of the peptids split off from the protein should be especially noted. From these data and the experiments of White and Crozier it is evident that the proteins studied break down into simple cleavage products practically as soon as they go into solution. At the end of a half hour's digestion the average size of the peptids is only 2.02; the cleavage products on the whole are indicated to be amino acids. The cleavage of certain proteins by trypsin has been intimately studied, and it is known that some amino acids are readily formed, while others are produced slowly or not at all. The above results, however, show that the greater portion of the nitrogen in solution exists in bodies of exceedingly simple character. The significance of this physiologically can not be pointed out here, but will be reserved for future discussion.

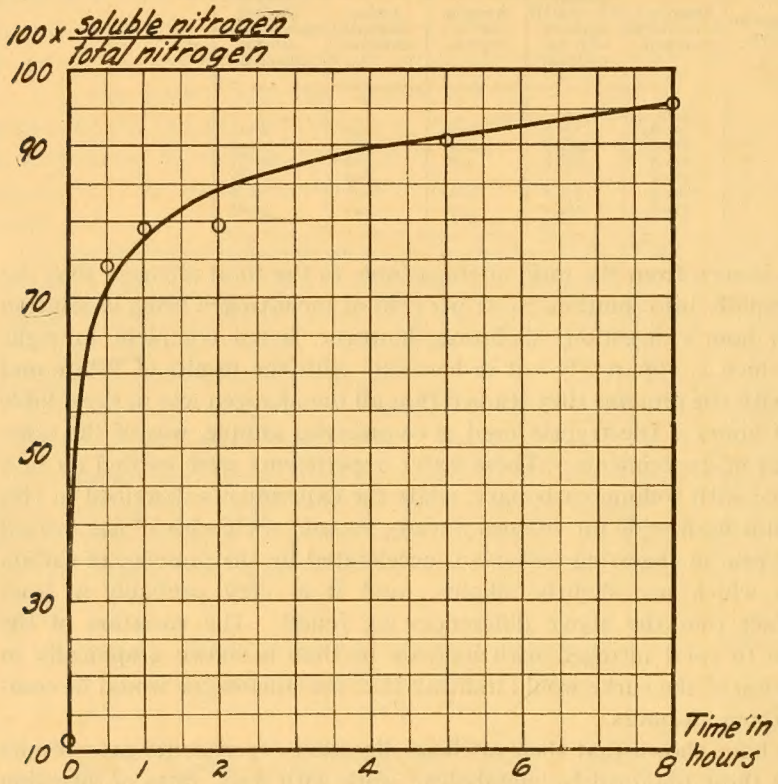

FIG. 1.—Change of the per cent of soluble nitrogen of the total nitrogen during the time of proteolysis.

The relation of the amino to the total soluble nitrogen is shown in figure 2. After eight hours' digestion only 28.35 per cent of the nitrogen is in the amino form. This is a confirmation of earlier work which, as just mentioned, has shown that there are certain substances which resist the hydrolytic action of trypsin altogether. The increase in the proportion of amino nitrogen during the eight hours' digestion is very slight, and we must conclude, therefore, that the cleavage of the meat, while yielding amino bodies of a simple nature, leaves the greater part of the soluble nitrogen combined in substances which are extremely stable.

The same conclusions may be drawn from the results of our experiments involving Sörensen's method. In figure 2 the curves are of the same slope, although, of course,

only approximate figures are expected on assuming the presence of one amino group for every carboxyl group indicated by the sodium hydroxide required. The amino nitrogen thus estimated is regularly greater than that determined by the nitrous acid method, and the peptids as computed are about 1.5 times as large. It is possible that in such calculations this effect is produced by the presence of such monamino-dicarboxylic compounds as glutamic acid. The discrepancy in the results is not of such a magnitude as to prevent deducing rigid conclusions concerning the rate and course of digestion of such proteins as the one under investigation.

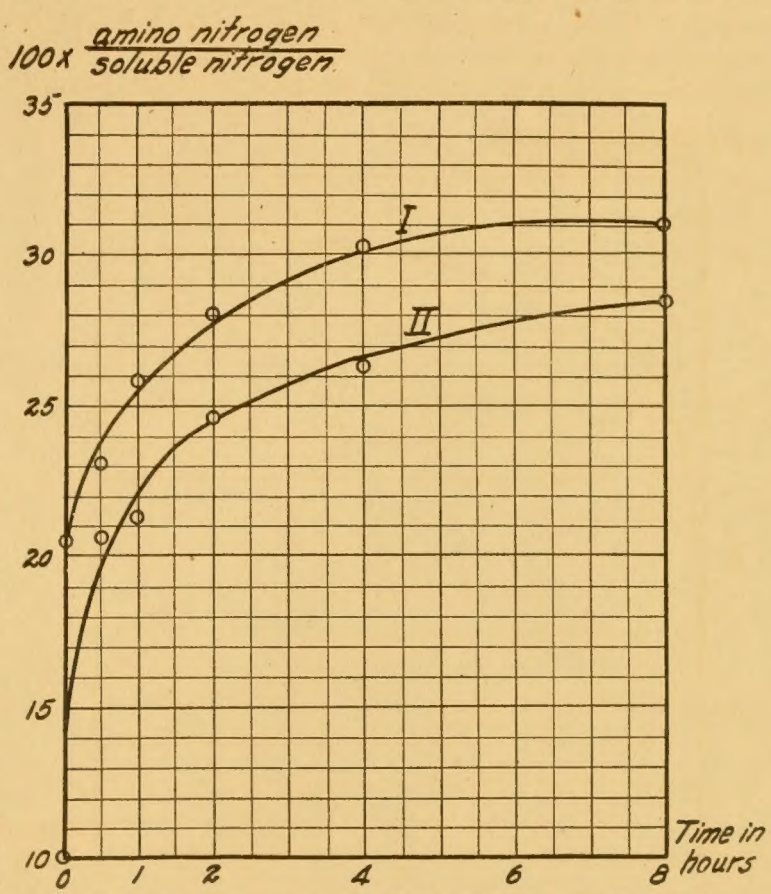

FIG. 2.—Change of the per cent of amino nitrogen of the soluble nitrogen during the time of proteolysis.

SUMMARY.

1. Sörensen's method for the determination of amino acids was applied to a study of the tryptic proteolysis of *Cynoscion regalis*. The results were regular and in accord with those obtained by the nitrous acid method for the analysis for amino nitrogen. A practical method for the determination of the food value of proteins has therefore been developed.

2. The relatively low rate at which the protein is made soluble agrees with the results of metabolism experiments.

3. Very low cleavage products are formed as soon as the protein goes into solution, the average size of the peptids being 2.02 after a half hour's digestion.

4. There is a very stable nitrogen complex which is not attacked by trypsin.